Roles of *Xylella fastidiosa* pili in motility and biofilm formation:regulation via a putative chemosensory system

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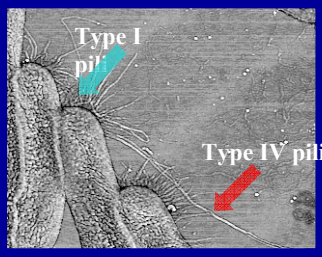
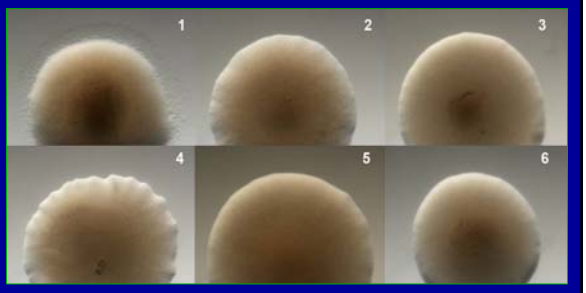
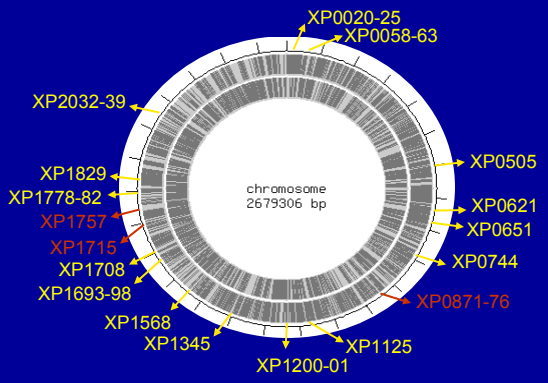




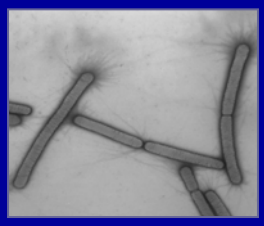
Genes Associated with Pili Biogenesis and Function in *X. fastidiosa*

At least 50 genes participate in the biogenesis and function of type I and type IV pili in *Xylella.*

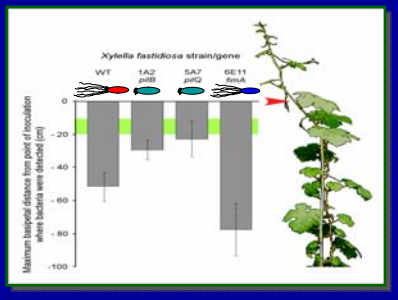


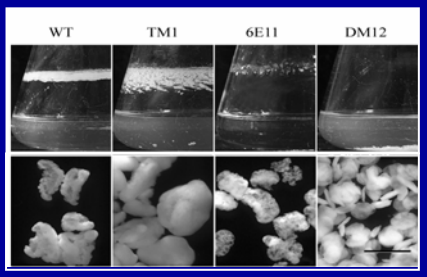
Modified from: http://gib.genes.nig.ac.jp/single/index.php?spid=Xfas_TEMECULA1

Type I and Type IV Pili Have Separate Functions



- Adhesion to surfaces
- Biofilm formation
- Speed of movement in plant
- Aggregation





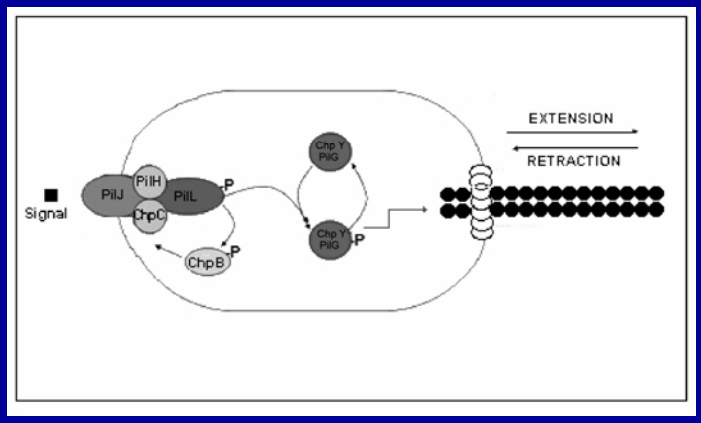
Regulation of Pili, Biofilm and Twitching Motility in *Xylella*

- *pilR*/*pilS* (Burr,Hoch group, Li et al. 2007)
  - Two component regulatory in several bacteria
  - Loss of motility and enhanced biofilm
- *rpf* (Lindow group, Newman et al. 2004)
  - Biofilm/vector relations and disease expression
- *rpnO* (Marques group, da Silva Neto et al. 2007)
  - Regulation of *pil* genes (*pilA1*) dependent on σ^{54}
  - Enhanced biofilm
- *gacA* and *algU* (Cooksey group, Shi et al. 2007)
  - Biofilm regulation
- Chemosensory cluster
  - Many bacteria, flagella and pili-mediated motility

Components of the Chemosensory Cluster of *X. fastidiosa*

Gene Id	Predicted protein	Synonym	Size (aa)	MW (kDa)	pI
XP0871	PilG	CheY	138	15	9.3
XP0872	PilI	CheW	176	19	6.1
XP0873	PilJ	MCP	680	72.7	5.6
XP0874	PilL	CheA	1725	190	4.7
XP0875	ChpB	CheB	389	41.4	4.5
XP0876	ChpC	CheW	156	17.1	4.6
XP1715	PilH	CheY	128	14	6.3
XP1757	ChpY	CheY	566	63.7	5.1

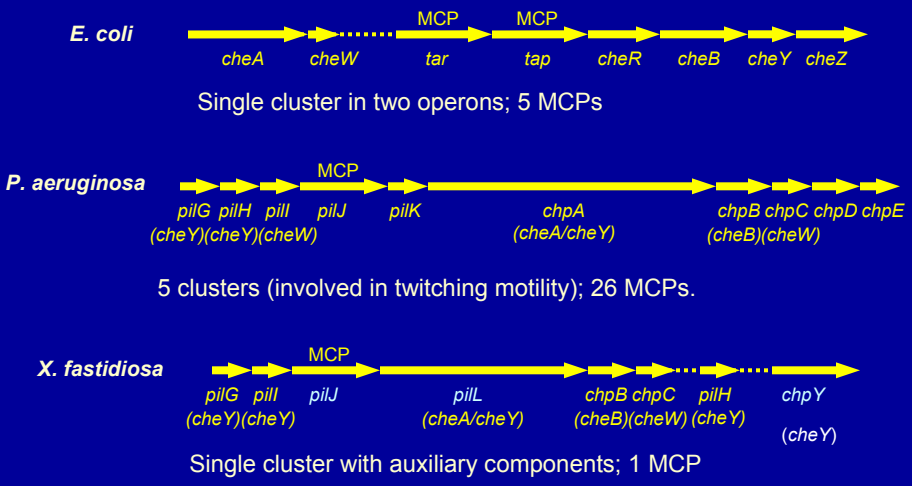
Model of Che Regulation in *Xylella*



PilJ (MCP)
PilH (coupling protein)
PilL (histidine kinase)

ChpY (response regulator) affecting pili extension/retraction?

Genomic Organization of Chemosensory Systems



PilL and ChpY are Essential for Twitching and Biofilm Formation

- The *pilL* mutant (XP0874) is twitching-minus and has a greatly reduced biofilm phenotype.
  - No cell movement in micro-fabricated flow chambers
- The *chpY* mutant (XP1757) is reduced in twitching and has a strongly-enhanced biofilm.
  - Reduced twitching motility in flow chambers
  - There are 20 proteins with CheY domains in *X. fastidiosa*.
- It was possible to complement the *chpY* mutant by cloning in pBBR1MCS-5.

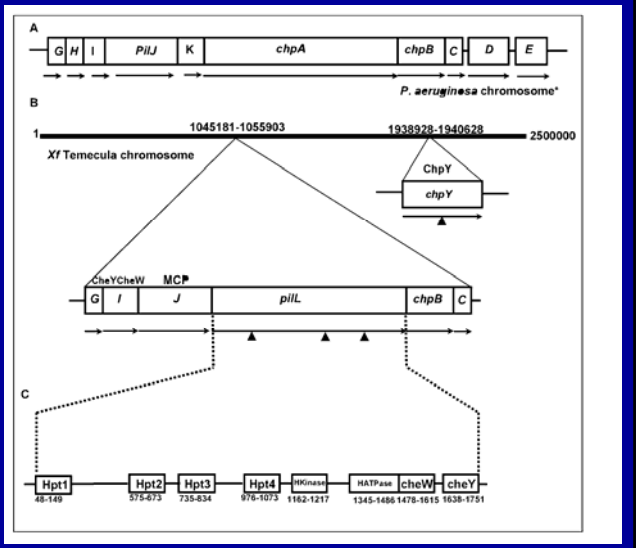
PilL (XP0874) is Characteristic of CheA Histidine Kinase

(HATPase) autocatalytic Histidine kinase domain for ATP binding

CheY docking domain

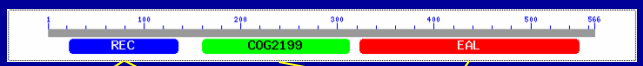
CheW mediates interaction With CheA

4 (Hpt) histidine-containing phosphotransfer domains



ChpY Structure and Putative Functions

Unusual protein highly conserved in *Xylella* and *Xanthomonas*:



3D modeling of CheY domain

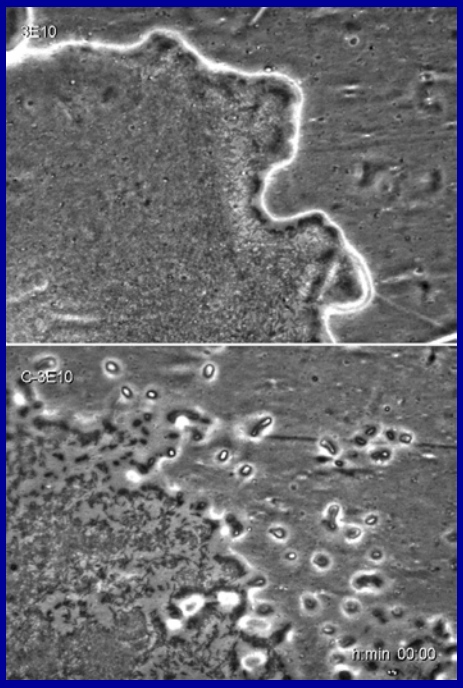
Domains involved in synthesis and degradation of cyclic di-GMP; second messenger associated with biofilm and other regulation in bacteria

ChpY: complex protein involved in the *ch*emosensory control of type-IV *p*ilus function and biofilm formation.

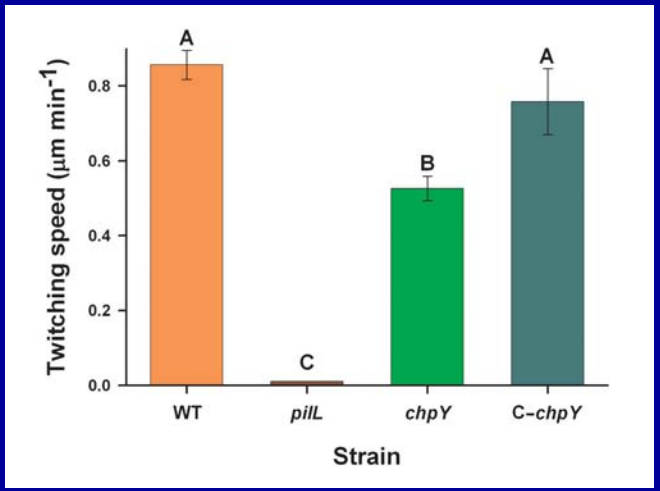
Motility Phenotype of *chpY* Mutant

chpY mutant

Complemented *chpY*



Effect of *pilL* and *chpY* on Speed of Twitching Movement in Micro-Fabricated Flow Chambers



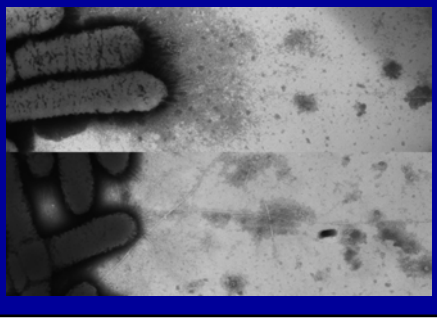
20 proteins with CheY domains; may partially compensate for loss of *chpY*

Effect of *pilL* and *chpY* on Pili and Colony Fringe

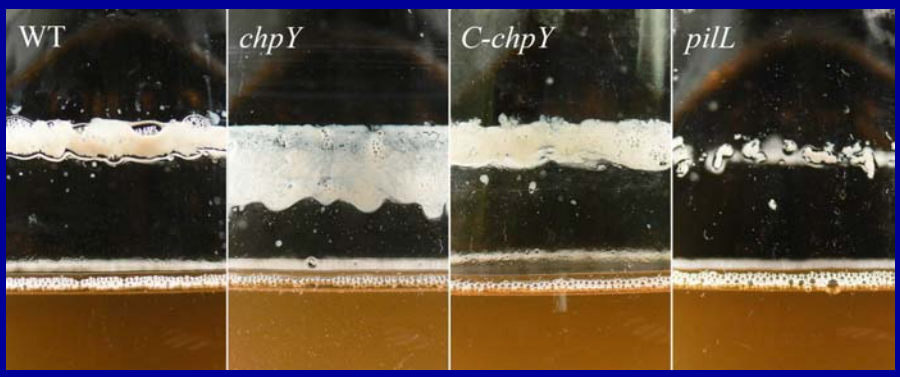
WT | *pilL* and *chpY* mutants | Complemented *chpY*

Both *pilL* and *chpY* mutants have type I and type IV pili.

*Whether pili numbers are reduced or enhanced remains to be determined

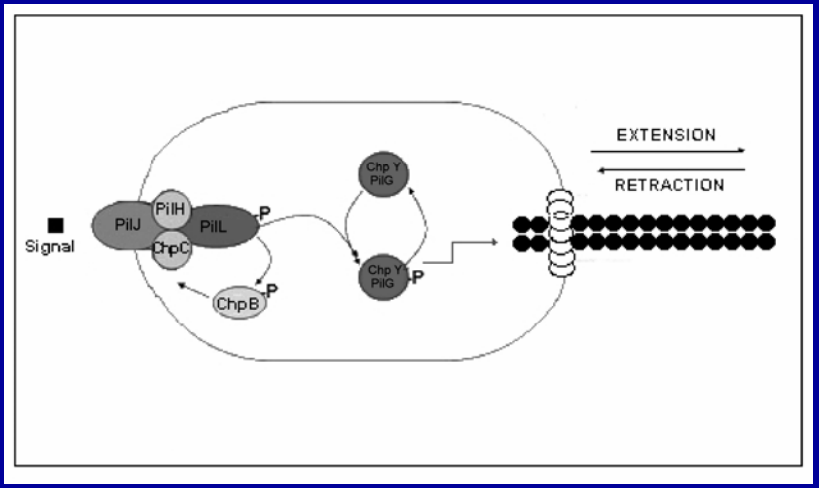


PilL and ChpY Affect Biofilm Production



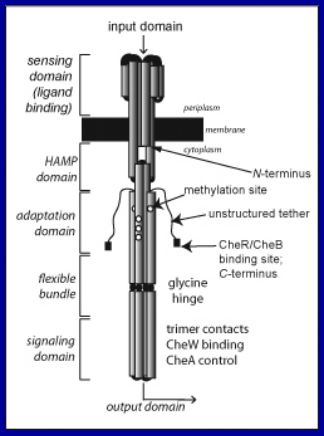
Loss of ChpY function greatly enhances biofilm. GGDEF and EAL domains affect c-di-GMP level; second messenger for biofilm regulation.

Model of Che Regulation in Xylella



X. fastidiosa has a single MCP, PilJ

MCP prototype



•MCPs (*m*ethyl-accepting *c*hemotaxis *p*roteins) initiate the cascade of signal transduction in response to diverse chemical and physical environmental stimuli.

•The occurrence of a single MCP in the genome of *Xylella* and its presence in the chemosensory cluster involved in motility calls for further investigations to identify the signal(s) responsible to trigger this cascade and affect motility.

Conclusions and Significance

- *X. fastidiosa* is unusual in that it has type I and type IV pili that play roles in biofilm and aggregate formation and in motility.
- *X. fastidiosa* employs type IV pili to move against transpiration stream in grapevines.

Conclusions and Significance

- Regulation of pili biogenesis, twitching and biofilm formation is complex and includes multiple systems.
- Understanding regulation of activities such as biofilm development and motility, that are believed to be critical to disease, offers possible targets for control strategies

Conclusions and Significance

- A chemosensory system in *X. fastidiosa* is essential for twitching motility and affects biofilm formation.
- Identification of components of the Che system and associated environmental signals may provide a novel means of disease control.
- Effect of Che knockouts on disease?